भारतीय मानक
Indian Standard

IS 17718 : 2022

इन-*विट्रो* डायग्नोस्टिक्स (आईवीडी) अभिकर्मकों/किट का प्रदर्शन परीक्षण — α-Amylase (सीएनपी-जी3), तरल स्थिर नैदानिक रसायन शास्त्र अभिकर्मक/किट

Performance Testing of *In-Vitro* Diagnostics (IVD) Reagents/Kits — α-Amylase (CNP-G3), Liquid Stable Clinical Chemistry Reagents/Kits

ICS 11.100.10

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भारतीय मानक ब्यूरो
BUREAU OF INDIAN STANDARDS
मानक भवन, 9 बहादुरशाह ज़फर मार्ग, नई दिल्ली – 110002
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI-110002
www.bis.gov.in www.standardsbis.in

October 2022 **Price Group 6**

Biological Evaluation of Medical Devices and *In-vitro* Diagnostic Medical Devices Sectional Committee, MHD 19

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards after the draft finalized by the Biological Evaluation of Medical Devices and *In-vitro* Diagnostic Medical Devices Sectional Committee and approval by the Medical Equipment and Hospital Planning Division Council.

This standard describes the specifications and procedure for estimation of α-Amylase present in human serum or plasma. The present method is based on the use of a chromogenic substrate, 2-chloro-p-nitrophenyl maltotrioside (CNP-G3). The reaction of Amylase with this substrate results in the formation of 2-chloro-p-nitrophenol, that can be measured photometrically. This reaction proceeds very rapidly, no coupling enzymes are required and the reaction is not readily inhibited by endogenous factors.

The composition of the Committee responsible for formulation of the standard is given in Annex B.

For the purpose of deciding whether a particular requirement of this standard is complied with the final value, observed or calculated, expressing the result of a test or analysis shall be rounded off in accordance with IS 2 : 2022 ‘Rules for rounding off numerical values (*second revision*)’. The number of significant places retained in the rounded off value shall be the same as that of the specified value in this standard.

Indian Standard

PERFORMANCE TESTING OF *IN-VITRO* DIAGNOSTICS (IVD) REAGENTS/KITS — α-AMYLASE (CNP-G3), LIQUID STABLE CLINICAL CHEMISTRY REAGENTS/KITS

1 SCOPE

1.1 This standard describes the specifications and standard testing procedure, for determining the performance of *In–vitro* Diagnostics (IVD) clinical chemistry reagents/kits, used for the estimation of α-Amylase in human serum or plasma, based on the CNP-G3 method.

1.2 This test method is recommended for all forms of α-Amylase (CNP-G3), liquid stable clinical chemistry reagents/kits except those used in dry chemistry analyzers and closed fully automated chemistry analysers.

2 REFERENCES

The standard(s) listed below contain provisions which, through reference in this text, constitute provisions of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on the standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below:

IS No.	*Title*
IS/ISO 23640 : 2011	*In-vitro* diagnostic medical devices — Evaluation of stability of *In-vitro* diagnostic reagents
IS/ISO 15190 : 2003	Medical laboratories — Requirements for safety

3 TERMINOLOGY

For the purposes of this Standard, the following definitions apply:

3.1 Accelerated Stability Study — In accelerated stability tests, a product is stored under specified, stressful conditions, such as elevated temperature and/ or humidity.

3.2 Controls — Laboratory quality controls are designed to detect, reduce and correct deficiencies in a laboratory's internal analytical processes, prior to the release of patients' results, in order to improve the quality of the results reported by the laboratory.

3.3 Deionized Water — Deionized water is a form of purified water, from which dissolved salts and minerals have been removed.

3.4 Kinetic Reaction — An enzyme-based reaction that measures the amount of substrate present by correlation of the rate of reaction with the known dependence of the rate on substrate concentration, usually under first-order conditions.

3.5 Linearity — The ability of a test to obtain results (within a given range) that vary in a manner directly proportional to changes in the concentration (amount) of the analyte in the sample or by a well-defined mathematical transformation.

3.6 Performance Check — A battery of tests recommended to ensure that the under-test reagent/kit, is accurately able to detect or measure the substance it claims to detect or measure and the measurement or detection of this substance provides important information about an illness or about health status that assists in the diagnosis, treatment, or monitoring of a patient.

3.7 Real Time Stability Study — Real-time stability testing is defined as monitoring and testing of a product stored at recommended storage conditions until it becomes unstable.

3.8 Specification — An act of identifying something precisely or of stating a precise requirement.

4 PRINCIPLE

Defined oligosaccharides, such as 2-chloro-4-nitrophenyl-α-D-maltotrioside are cleaved under the catalytic action of α-Amylases.

The release of the chromogen, CNP, is then measured photometrically during a kinetic reaction as shown below:

2-Chloro-4-Nitrophenyl-α-D Maltotrioside

$$\Big\downarrow{\scriptstyle \alpha\text{-Amylase}}$$

α-Chloro-Nitro Phenol (CNP) + CNP-G2 + 9G3 + G

The intensity of the chromogen CNP formed, is directly proportional to the α-Amylase activity in the sample being tested and is measured kinetically, at 405 nm using a photometer.

NOTE — The method generally obeys the Beer's law up-to a level of 2 000 IU/L of α-Amylase activity in the sample.

α-Amylase activity may be measured in IU/L or µkat/L

Conversion from IU/L to microkat/L: 1.0 IU/L = 0.017 µkat/L

5 MATERIALS

5.1 Chemicals

a) α-Amylase (CNP-G3), liquid stable reagents/kit (under test);
b) Deionized water;
c) α-Amylase linearity level (2000 IU/L) or as per the claim of the manufacturer;
d) Commercially available control/s of validated quality, normal range;
e) Commercially available control/s of validated quality, abnormal range; and
f) Clinical samples, normal and abnormal. Sample size provided should be appropriate and sufficient to render the study statistically significant.

5.2 Apparatus and Equipment

a) Photometer;
b) Incubator;
c) Test tubes;
d) Test tube stand
e) Micropipettes (5 to 50 µL; 50 to 200 µL; 200 to 1000 µL); and
f) Disposable tips for micropipettes.

6 WORKMANSHIP AND FINISH

After manufacturing, the α-Amylase reagents/kit shall be converted into a finished product as under:

6.1 Reagents should be filled as per the pack size of the manufacturer in leak proof, compatible bottles/containers.

6.2 The filled, capped, labeled (FCL) bottles should be placed in an appropriate size of carton using partitions to avoid rattling during transportation.

6.3 Quality seals should be affixed on the packed carton after final quality checks by the manufacturer to avoid pilferage.

6.4 Instructions for use in English Language shall be accompanied with the package.

7 TEST PROCEDURE

7.1 Visual Inspection

7.1.1 Inspect the under-test kit. It should be compliant with the manufacturer's specifications.

7.1.2 It should be appropriately labeled.

7.1.3 It should have been appropriately stored between 2 to 8 °C.

7.2. Performance Check (Kinetic Reaction)

The procedure below is provided as an illustration. Follow the protocol as per the claims and specifications of the manufacturer.

7.2.1 Take the kit under-test.

7.2.2 Take 25 clean and dry reusable test tubes (5 to 10 mL capacity).

7.2.3 Mark 2 to 6 tubes for controls (normal/abnormal), 2 to 10 tubes for samples (normal/abnormal), 1 to 6 tubes for linearity/levels (100, 200, 400, 800, 1000, 2000 IU/L).

7.2.4 Pipette 1.0 mL of the α-Amylase Reagent into each test tube or as per the protocol mentioned in the manufacturer's product manual.

7.2.5 Add 25 µL of control/samples/linearity levels, into the respectively labeled test tubes or as per the protocol mentioned in the manufacturer's product manual.

7.2.6 Mix gently for a few seconds and aspirate the reaction mixture in each tube or as per the protocol mentioned in the manufacturer's product insert, using a photometer.

7.2.7 After 1 min incubation at 37 °C, measure the change in absorbance/minute for another 2 min for each tube, at 405 nm or as per the protocol mentioned in the manufacturer's product insert.

7.2.8 Use this change in absorbance/minute for calculating the α-Amylase concentration of controls, samples and linearity levels.

7.2.9 The values obtained should be within the specified ranges as described by the manufacturer.

7.2.10 *Programming of the Photometer*

Refer to the following input parameters as indicated in Table 1 or as per the protocol mentioned in the photometer manufacturer's product manual.

Table 1 Input Parameters for Programming of Photometer

(*Clause* 7.2.10)

Input Parameters	Values
Type of reaction	Kinetic
Slope of reaction	Increasing
Wavelength	405 nm
Factor	3178
Incubation time	60 s
Interval time	60 s
Interval number	2
Units	IU/L
Temperature	37°C
Upper normal value	140 IU/L
Lower normal value	25 IU/L
Linearity	2000 IU/L
Working reagent volume	1.0 mL
Sample volume	25 µL

7.3 Calculations

α-Amylase concentration in IU/L maybe calculated as change in absorbance/minute × factor or as per the protocol mentioned in the manufacturer's product manual.

8 SPECIFICATIONS

α-Amylase (CNP-G3), liquid stable clinical chemistry reagents/kits shall be as per contents mentioned in the Table 2 or as per the specifications declared by the manufacturer.

9 TEST REPORT FORMAT

The test report may include the following information, but not limited:

a) Analytical report number;
b) Name of the under-test kit/sample to be tested;
c) Date of receipt of kit/sample;
d) Quantity of the kit/sample;
e) Batch/lot number;
f) Date of manufacturing;
g) Date of expiry; and
h) List of tests to be conducted as given in Table 3.

10 MARKING AND LABELLING

Each bottle/container of the α-Amylase reagents/kit shall display a label which contains all the information, in compliance with the regulatory requirements[1)], but not limited to the following:

a) Product name;
b) Reagent name;
c) Major ingredients;
d) Pack size;
e) Intended use;
f) Storage temperature;
g) For *In-vitro* diagnostics use only;
h) Batch No./Lot No.;
j) Manufacturer's complete name and address;
k) Marketer's (if any) complete name and address
m) Manufacturing date;
n) Expiry date;
p) Maximum retail price in Indian Rupees;
q) Precautions/cautions; and
r) Relevant graphical symbols.

11 BIS CERTIFICATION

The product(s) conforming to the requirements of this standard may be certified as per the conformity assessment schemes under the provisions of the *Bureau of Indian Standards Act*, 2016 and the Rules and Regulations framed thereunder, and the product(s) may be marked with the Standard Mark.

12 DISPOSAL OF EMPTY VIALS/REAGENTS AFTER TESTING

Disposal of empty vials/reagents after testing as per the requirements of IS/ISO 15190 Medical laboratories — Requirements for safety.

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[1] Medical Device Rules, 2017.

Table 2 Tests Parameters

(*Clause* 8)

Sl No.	Tests/Parameters	Specifications
1.0	**Description**	For the *In-vitro* estimation of α-Amylase concentration in human serum or plasma.
2.0	**Kit contents**	
2.1	Reagent	1 × 10 mL or as per manufacturer's pack presentation
2.2	Package insert	01 no. or as per manufacturer's pack presentation
3.0	**Packing material/kit**	
3.1	10 mL, plastic bottle with cap	OR as per manufacturer's packaging specifications
3.2	Label for bottle	as per manufacturer's packaging specifications
3.3	Package insert	as per manufacturer's packaging specifications
3.4	Carton	as per manufacturer's packaging specifications
3.5	Label for carton	as per manufacturer's packaging specifications
3.6	Shrink sleeve for carton to protect from moisture	as per manufacturer's packaging specifications
4.0	**Reagent volume/kit**	
4.1.	Reagent 1	10 mL + 0.1 mL overage or as per manufacturer's specifications
5.0	**Performance check (on day-0)**	
5.1	Initial absorbance	0.800 max or as per manufacturer's specifications
5.2	Controls	At least 2 out of 3 controls' results should be within specifications as per supplier's value sheet. Controls used should be from a traceable inter laboratory comparison study.
5.3	Linearity	2000 IU/L ± 10 percent or as per manufacturer's specifications.
5.4	Clinical samples	Normal Range for clinical samples 25 to 140 IU/L or as per manufacturer's specifications.
6.0	**Real time stability study (optional) (Annex A)**	The Performance of the Kit measured after 6, 12, 18 months, at expiry and 3 months after expiry, should be ± 10 percent of the day-0 performance.
7.0	**Storage and stability**	The reagent should be stored in 2 to 8 °C. The reagent should be stable till the expiry date mentioned on the label. Do not freeze the reagent.

Table 3: List of Tests to be conducted

[*Clause* 9 (h)]

Sl No.	**Parameters**	**Test Results**
1	Description	
2	Visual inspection	
3	Initial absorbance	
4	Results obtained with linearity levels: 100 IU/L 200 IU/L 400 IU/L 800 IU/L 1000 IU/L 2000 IU/L	
5	Results obtained with commercial controls (controls should be from a traceable inter laboratory comparison study): Set 1 (low, normal, high) Set 2 (low, normal, high) Set 3 (low, normal, high)	
6	Results obtained with clinical samples Sample size selected should be appropriate and sufficient to render the study statistically significant: Normal Abnormal	
7	Results obtained with other tests as recommended by the manufacturer.	

NOTE — Records regarding various tests conducted (including readings and calculations) should be maintained and reference to these records should be entered whenever necessary.

ANNEX A

(*Table* 2)

A-1 REAL TIME STABILITY STUDY TO BE CONDUCTED AS PER STANDARD IS/ ISO 23640

A-1.1 The same kit as in **5.1** a) can be used for all the following studies.

A-1.2 If the kit under test passes all the above tests as specified in **7.1** to **7.2**, keep the kit at 2 °C to 8 °C for the following below mentioned time points: Sampling time points: 6, 12, 18, at expiry and 3 months after expiry.

A-1.3 Perform the check on the kit as per **7.2**.

A-1.4 The performance of the reagent should be as per specifications.

ANNEX B

(*Foreword*)

COMMITTEE COMPOSITION

Biological Evaluation of Medical Devices and *In-vitro* Diagnostic Medical Devices Sectional Committee, MHD 19

Organization	*Representative(s)*
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Panacea Biotec Limited, New Delhi	Dr Harish Chandra Shri Anil Sood (*Alternate*)
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Sensa Core Medical Instrumentation Pvt Ltd	Mr Pavan Chandra Nagoor Naralasetty
Siemens Healthcare Pvt Ltd, Gurugram	Shri Manish Ragtah
Span Diagnostics Limited, Surat	Dr Prabir Kumar Ghosh

Organization	*Representative(s)*
Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum-Biomedical Technology Wing	Dr Anoop Kumar Dr Manoj Komath (*Alternate*)
Stryker India Private Limited, Gurugram	Mr Shivkumar Hurdale
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Ms Nagavarshini M.
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BUREAU OF INDIAN STANDARDS

Headquarters:

Manak Bhavan, 9 Bahadur Shah Zafar Marg, New Delhi 110002
Telephones: 2323 0131, 2323 3375, 2323 9402 *Website*: www.bis.gov.in

Regional Offices:		*Telephones*
Central	: 601/A, Konnectus Tower-1, 6th Floor, DMRC Building, Bhavbhuti Marg, New Delhi 110002	2323 7617
Eastern	: 8th Floor, Plot No 7/7 & 7/8, CP Block, Sector V, Salt Lake, Kolkata, West Bengal 700091	2367 0012 2320 9474
Northern	: Plot No. 4-A, Sector 27-B, Madhya Marg Chandigarh 160019	265 9930
Southern	: C.I.T. Campus, IV Cross Road, Taramani, Chennai 600113	2254 1442 2254 1216
Western	: Plot No. E-9, Road No.-8, MIDC, Andheri (East), Mumbai 400093	2821 8093

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